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Concomitant p53 Mutation and MYCN Amplification in Neuroblastoma

Reinaldo Manhani,[1] Lilian Maria Cristofani, MD, PhD,[2] Vicente Odone Filho, MD, PhD,[2] and Israel Bendit, MD[1]*

The MYCN oncogene is amplified in 20% of childhood neuroblastoma and is associated independently with poor prognosis. Alteration of the p53 tumor supressor gene, in contrast, occurs infrequently in these tumors. In this report, we described a 3-year-old girl with stage IV neuroblastoma. Molecular analysis revealed both MYCN gene amplification and a point mutation of the p53 tumor supressor gene. To our knowledge, this is the first reported case of neuroblastoma with genetic alterations of both these genes. Med. Pediatr. Oncol. 29:206–207, 1997. © 1997 Wiley-Liss, Inc.

Key words: neuroblastoma; p53 mutations; MYCN gene amplification

INTRODUCTION

Neuroblastoma (NB) is the most common extracranial solid tumor of childhood [1]. Studies on MYCN oncogene amplification as a prognostic marker of poor outcome in these neoplasms have served as a paradigm for translational studies [2]. Additional genetic alterations that occur in neuroblastoma and which have also been correlated with poor outcome include LOH for chromosomes 1 and 14, trisomy of 17 q, and low expression of TRK-A [3–11].

Mutation of the p53 tumor supressor gene on chromosome 17p13 is the most frequent genetic alteration in human cancers to date [12,13]. However, in neuroblastoma, as in many pediatric solid tumors, p53 mutations occur infrequently [14–18]. In this report we describe a case of stage IV NB in which both MYCN amplification and point mutation of the p53 tumor supressor gene were identified.

MATERIAL AND METHODS

Case Report

A 3-year-old girl presented with right adrenal and periorbital masses, bone pain, pallor, and fever. Bone marrow analysis revealed infiltration with neuroblastoma. Bone lesions were diffuse and CT revealed meningeal involvement. She was treated with intensive chemotherapy (Carboplatin, Epipodophylotoxin, Cyclophosphamide, Doxorubicin) and surgery. She eventually died of progressive disease.

Molecular Analysis

High molecular weight DNA was extracted from tumor samples obtained at the time of diagnosis and second-look surgery following chemotherapy using proteinase as described elsewhere [19]. MYCN amplification was measured by Southern blot analysis as described previously [19]. p53 mutations were identified by direct nucleotide sequence analysis of PCR products using Sequenase™ (AMERSHAM International, Buckinghamshire, UK), oligonucleotide primers, and conditions described elsewhere [20].

RESULTS AND DISCUSSION

As part of a protocol designed to determine prognostic markers in NB, MYCN gene amplification in the tumor specimen was determined by Southern blot analysis. The tumor specimen at diagnosis and after chemotherapy contained more than 200 copies of the MYCN oncogene. This finding predicted a poor outcome for the patient, which was unfortunately confirmed when she died 9 months after diagnosis despite intensive chemotherapy.

The tumor specimen from both occasions was also tested for mutation of the p53 tumor supressor gene by direct DNA sequence analysis of exons 5-8. A G→T transversion was identified (Cys→Phe) at codon 277 that alters the charge of the amino acid (Fig. 1) in the tumor specimen obtain at second-look surgery after chemotherapy; p53 mutations were not detected in the tumor sample obtained at the time of diagnosis (or in the blood from the patient, confirming that this is a de novo mutation and not a polymorphism). This exact mutation has not been described before, but similar alterations at this codon, which is a hotspot for mutation, have been de-

[1]Research and Molecular Biology Division, Pró-Sangue Hemocentro de São Paulo Foundation, São Paulo, Brazil; [2]Children's Institute of the University of São Paulo Medical School, São Paulo, Brazil

*Correspondence to: Israel Bendit, M.D., Research and Molecular Biology Division, Pró-Sangue Hemocentro de São Paulo Foundation Av. Dr. Enéias de Carvalho Aguiar, 155 1st floor CEP 05403-000, São Paulo, Brazil.

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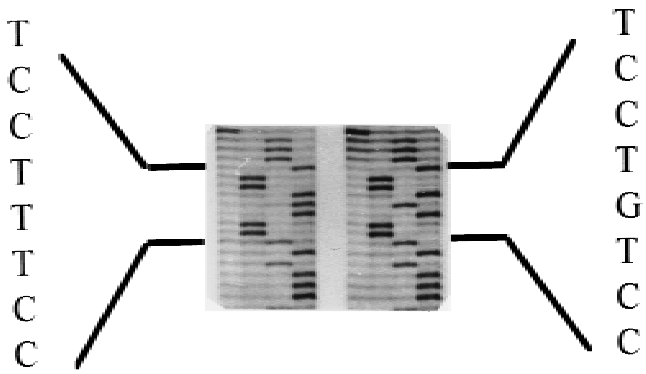

Fig. 1. DNA sequence of p53 exon 8 in DNA from neuroblastoma specimen which exhibited anomalous migration on SSCP analysis (data not shown). p53 exon 8 DNA including codon 276 and 278 from neuroblastoma obtain at second look is at left. Wild-type p53 exon 8 DNA sequence from neuroblastoma at diagnosis is at right.

scribed [21,22]. The p53 mutation, which occurs at a CpG dinucleotide, may have been present but undetected in a small subset of original tumor cells that subsequently expanded, leading to its later identification in the second-look surgical specimen. Alternatively, the mutation could have arisen de novo as a result of the chemotherapy. In either case, detection of p53 mutation was associated with a progressive tumor that was unresponsive to chemotherapy. p53 mutations can be associated paradoxically with either increased or decreased sensitivity to chemoradiotherapy [23].

There are several possible explanations for the infrequency of p53 mutation in NB as compared to the prevalence in other common solid tumors in childhood [24–28]. It is possible that other mutations (e.g., deletions on chromosome 1 or 14) affect the pathway.

While other studies confirm the frequency of MYCN amplification and infrequency of p53 mutations in neuroblastoma [14–18], to our knowledge, this is the first case in which both genetic alterations were observed to occur in the same tumor.

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